MIC 401: RESEARCH PAPER

Disinfection of Giardia lamblia Contaminated water using Titanium Dioxide and UV Radiation

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Solar disinfection of water is method widely known and widely used in developing countries. This technique involves placing water into transparent 2 Liter plastic polyethylene bottles, and then exposing them to the sun exposure time varies from 6 to 48 hours based on sunlight intensity and water turbidity (1). Using Titanium Oxide films in the bottles and exposing them to the sun has shown to be more effective that UV radiation alone. It has been shown to be effective in eliminating microbial pathogens to reduce cases of Giardiasis. Water samples would be tested after solar exposure, to ensure eradication of Giardia cysts. After ensuring no cysts are present in water samples, mouse models would be injected with the water samples to test for Giardia antigen presence in feces. Results from this method would ensure a safe and cost

effective way to disinfect drinking water in developing countries. Not only would Giardiasis cases decrease, but cases of malnutrition and other illnesses due to giardiasis would also be decreased.

Background and Introduction

Water is a precious commodity. It is necessary for human survival. Many people go about their days without giving the abundance of clean water a second though. Developed towns and cities provide us with clean water because they have the means and infrastructure to clean and disinfect that water. There are places where clean water is a luxury. The United Nations International Children's emergency Fund (UNICEF) and the World Health Organization (WHO) estimate that in 2011, 2.5 billion people did not have access to safe drinking water (2). Water can be contaminated with several types of water-borne pathogens including *Vibrio cholera*, *Shigella dysenteriae,* and *Giardia lamblia.*

Giarda cysts are transmitted through fecal-oral route. Some mechanisms of infection include person to person, animal to animal, and animal to person, waterborne through drinking water or recreational waters. There had been more than 100 waterborne giardiasis outbreaks worldwide since 1900 till 2004 (3). As of 2010, the largest drinking water outbreak had occurred in 2004. Giardiasis is an intestinal infection caused by *Giardia lamblia*. It is most prevalent in areas of poor sanitation and unsafe water. Research suggests that *Giardia* induced diarrheal disease is mediated by small intestinal malabsorption and mal-digestion. Clinical signs of infection include nausea, weight loss, bloating, abdominal pain, and diarrhea (4).

Giardia lamblia is rarely fatal. But when it is fatal, it is by causing dehydration and extreme weight loss in infants, young children, and immunosuppressed individuals in developing countries. Halliez and Buret find that human giardiasis ranges from 20 to 30 percent in developing country populations, with some countries reporting 100 percent (5, 6). Studies were conducted on children in Brazil and Peru and concluded that giardiasis occurring in the first two years of life have a negative correlation with cognitive function, verbal fluency, physical fitness, and possibly growth faltering (7, 8, 9). The infection is easily treated with antibiotics. These antibiotics have bad side effects, and reoccurrence of the disease is common in the developing world.

Because the *Giardia* causing the disease is found in drinking water, one way to ensure their eradication is to disinfect the water. Methods of water disinfection differ from region to region. Of course, countries with more money and more infrastructure ensure their drinking water is accurately disinfected enough to distribute. That is not the case for those living in developing countries. Those that get their water from streams, wells, and rivers in developing countries are likely to contract giardiasis, due to contamination of water through runoff.

Giardia cysts are the stage that is infective in G. *Lamblia.* It takes as little as 10 cysts to cause infection (10). Ingestion takes place by consumption of contaminated food as well as water, or fecal-orally. These cysts are able to survive outside of the human body for months on end, and are resistant to chlorine, as well as well as UV light and extreme cold.

After cysts are ingested, the acidity of the stomach produces excystation, which allows the flagella to break through the hard cyst wall (10). This process takes place in the duodenum, in

the small intestine. The results of this process include release of 2 trophozoites per cyst (11). While in the small intestine, asexual reproduction by binary fission, takes place. The trophozoites attach themselves to the mucosa of the lumen (11).

Some trophozoites go through the process of encystation in the small intestine (11). This happens when they are exposed to a more alkaline environment (12). Both trophozoites and cysts are then passed in the feces, and become infectious shortly after (11).

According to the Environmental Protection Agency (EPA), the best way to disinfect water is to first filter the water and then boil it. Let the water cool, and then transfer it to a clean container. To treat the water chemically, the water should first be filtered and then unscented household bleach used. Both iodine tinctures and calcium hypochlorite are better at treating contaminated water than bleach is. However, there are significant safety issues when using these chemicals (13).

Living in a developing nation, people might not have the resources to perform those procedures. Fuel for boiling water might not even be available. In this situation, a water disinfection technique that is cost effective is Solar Disinfection (SODIS). This process takes advantage of sunlight and polyethylene terephthalate (PET) bottles. The SODIS process is simple to follow. Using PET bottles fill them ¾ full with water that is not too turbid. Turbid water protects pathogens from the sun's radiation. After shaking the bottle to aerate it, more water is added to fill the bottle. After leaving it in the sunlight for at least 6 continuous hours, the water should be disinfected (13).

A study was conducted by Wayne Heaselgrave and Simon Kilvinton on the antimicrobial activity of simulated SODIS in the presence and absence of riboflavin against various protozoa, including *Giardia* (14). Plates with test organism and riboflavin were exposed to simulated sunlight with a radiance of 550W per square meter delivered from a solar stimulator. Aliquots of the test organisms were taken at different time points and the presence of each organism was determined using culture microscopy or flow cytometry (14). With *Giardia*, exposure to SODIS as the designated optical irradiance for up to 6 hours resulted in significant deactivation of the organism (15).

Despite the accuracy of this technique the conditions simulated cannot be simulated in real like every day. It would be beneficial for communities and small town governments to monitor water safety by performing mass disinfection of water before distribution. A study was performed by Silvia Gelover and others at the Mexican institute of technology demonstrating water disinfection using TiO_2 films and sunlight. The researchers exposed spring water naturally polluted with coliform to sunlight with and without TiO_2 over simple solar collectors and the disinfection effectiveness measured. They gathered that the disinfection with TiO_2 was more efficient than SODIS. It took less than half the time for TiO_2 to disinfect the water than SODIS alone. After SODIS, bacterial regrowth was present. After disinfection with TiO_2 catalyst, no regrowth was observed (15).

Disinfection by titanium dioxide is based on its ability to kill bacteria upon reaction with light in aqueous solution (16). Its effects could be due to the generation of reactive oxygen species

generated by TiO_2 illumination. It is though that the decomposition of cell membrane by the photo-catalytic reaction is the cause of cell death (17, 18).

This method of disinfection opens up possibilities for water disinfection in rural areas of developing countries. If mass disinfection of water can take place, distribution of clean water would be more possible. Disinfection of water is important because it decreases the cases of water-borne pathogenic infections that take place. Cases of Giardiasis would be less prevalent. Future studies involving giardiasis and its methods of infection would include stopping its mechanism of infection in the human body by adding agents to the water that work against *Giardia lamblia*'s mechanism of infection once it makes contact with the small intestine.

Experimental Design

Method of cultivation (19)

TYI-S-33 medium preparation: each 100ml of medium contained the following

100mg K_2HPO_4

60mg KH_2PO_4

2 g trypticase

1.0 g yeast extract

1.0 g glucose

200 mg NaCl

200 mg cysteine-HCI monohydrate

20mg ascorbic acid

2.28mg ferric ammonium citrate

50-100 mg dehydrated bovine bile

10 ml of inactivated bovine serum (pH: 7.0 -7 .2)

The medium should be sterilized by passing it through a 0.45 µm membrane filter, and then supplemented with 15% bovine serum, penicillin (400 JU/ml), streptomycin (500 µg/ml), and gentamicin (50 µg/ ml). (19).

Axenic culture preparation

G. lamblia cysts should be obtained from Waterborne Inc. and 10^7 cysts should be stored in 8ml of phosphate-buffered saline (PBS) with 2.5% formalin at 4ºC. These cysts should be from an H-3 human isolate after passage through Mongolian gerbils (19).

The medium should be dispensed into tubes at 80% capacity that can be used for 7 to 10 days at 4 º C.

A 10^{-1} dilution of 0.01N HCL and cysts should be prepared. The preparation should then be incubated at 37ºC for one hour. Then centrifuged at 600rpm for 10 minutes. After centrifugation, the supernatant should be removed and the pellet sample washed with distilled water.

10^5 cysts should be inoculated into 13X100 mm screw- top borosilicate tube that contains TYI-S-33 culture medium containing bovine bile and bovine serum. After the trophozoites develop, the medium should be replaced every 2 to 4 days. When trophozoites grow, there should be a dense monolayer on the surface of the tubes' walls. To dislodge the monolayer, the tubes should be immersed in an ice water bath for 10 minutes, and then shaken for a few seconds. 1-2 ml of the trophozoite suspension should then be inoculated into a tube of fresh medium (19)

Preparation of TiO_2 thin films should be performed as stated in (20)

To prepare TiO_2 thin films, a 5mm diameter line of black silicone adhesive bathroom sealant like Evo-stick should be laid down one side of an A4-size overhead projector acetate transparency sheet (5star Ltd, Cambridge UK). A rectangular-tooth edged tilting trowel should be used to spread the adhesive across the plastic sheet in straight parallel lines.

Each sheet should then be covered with TiO_2 powder and allowed to dry for 24 hours. Excess TiO_2 powder should be shaken off and gathered. The sheets should then be washed with distilled water and left to dry overnight. The finished sheet should then be cut into proper dimension to cover the lower half of the PET bottles. The coated acetate insert should be rolled up and inserted through the neck of the bottles before being unfolded so place the TiO_2 coating facing the bottle's center.

Preparing water samples (20)

20µL of trophozoite suspension should be inoculated into 2L PET bottles along with 1 TiO_1 film and (mL) of distilled water and mixed well. The bottles should be placed on a flat surface under

the sun for 6 to 12 hours. After removal from the sun, the samples should be stored in the refrigerator until their use.

Cyst detection in water samples (3, 21, and 22)

The quantitative immunofluorescence assay should be developed using the commercial MERIFLUOR Cryptosporidium-*Giardia* kit from Meridian Diagnostics, Inc.

10ml of the water sample should be transferred to a 50 ml centrifuge tube and centrifuged at 1500 g for 10 minutes. The supernatant should then be decanted and the pellets dissolved to a total of 4ml by adding DI water. After vortexing, 20µL of the suspension should be pipetted onto a treated FA slide and stained with the FA kit as described in the kit directions (fig. 1) (22). After staining, the smear should be searched at a 100X magnification under a fluorescence microscope for *Giardia* cysts.

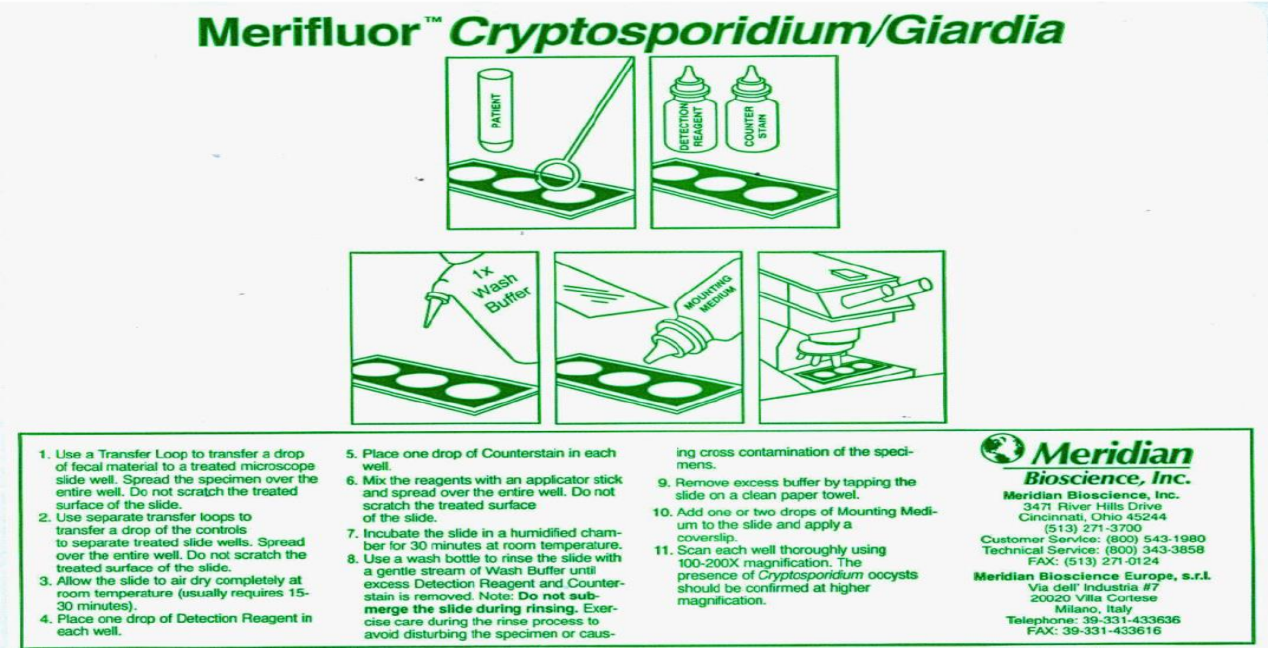

Figure 1: Merifluor *Giardia* testing in water samples

Infecting Mice with water samples

After the samples have been refrigerated, 10, 20, and 50µL of the water should be injected into the mouse models. For six (6) weeks, mouse feces should be tested daily for the first week and weekly after that, for detection of *Giardia* cyst wall protein 1 (CWP1) according to Techlab's *Giardia* II ELISA specimen preparation and procedure (23, 24).

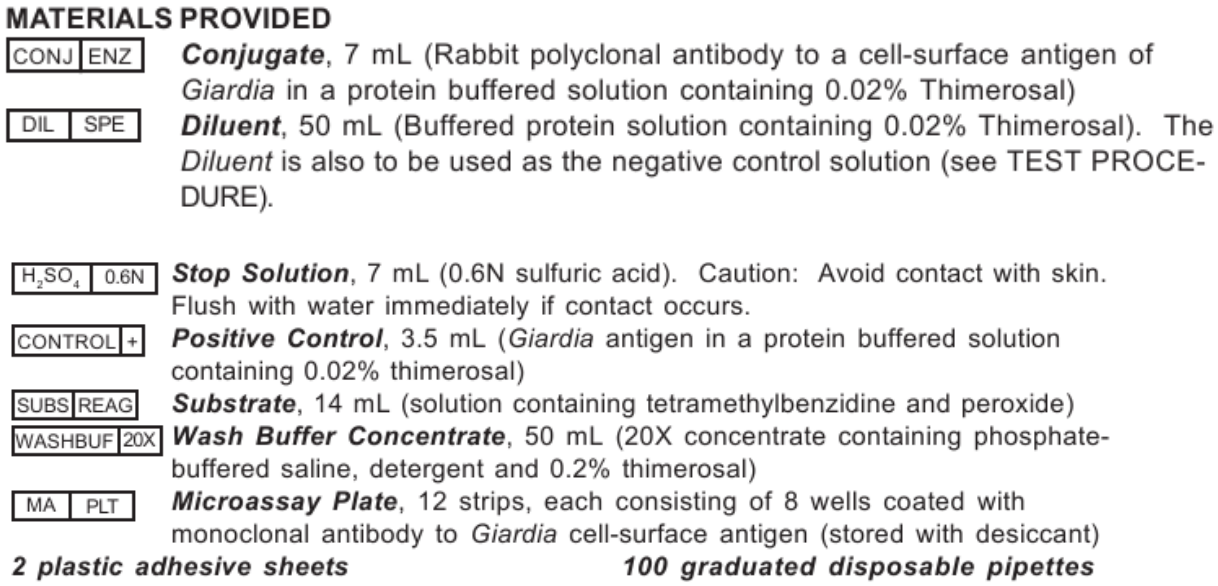

Figure 2: Materials Provided in Kit

*micro-pipettes can replace disposable pipettes

Materials required, but not provided (24)

Micro pipettes

1L distilled water for diluting wash reagent

Paper towels

Applicator sticks

ELISA reader

Vortex mixer

Biohazard container

Micro-centrifuge tubes

PROCEDURE

Specimen Preparation (24)

Prepare a 1:5 dilution

400µL of diluent should be added to a microcentrifuge tube containing a sample (1 per sample). Then, 100µL of sample should then be added to tube and well mixed. If the sample cannot be pipetted, .1 grams of feces should be added to the water to make a 10^{-5} dilution. Final dilutions should be performed in microassay wells as directed below.

Two (2) control wells (+ and -) must be used each time the test is performed. 50µL of the positive control solution should be added to the positive control well and 100µL of the negative control should be added to the negative control well.

100µL of diluent should be transferred to each test well on the plate and 50µL of sample should be added to each test well, which already contain diluent. Tap the wells to mix, and seal with plate sealer to incubate for 1 hour at RT.

After incubation, contents of the wells should be shaken off in biohazard container. Wells should be washed using the 1X wash solution using a micropipette. The wells should be filled and the wash a total of 4X and solution shaken out of the well onto the paper towel with a hard slap each time.

After washing, any residual liquid should be removed by striking the plate against the paper towel until dry. 50µL of Conjugate should then be added to each well and mixed. The plate should be sealed and incubated at RT for 30 minutes.

The washing procedure should be repeated. 100µL of substrate should then be added to the wells, mixed, and incubated for 10 minutes at RT. After incubation, stop solution should be added to the wells, and mixed. After two minutes, plates can be read by a microplate ELISA reader, measuring absorbance at 450nm. If using a dual reader, the reader should be blanked against air at 620nm and read at 450nm. Adding the stop solution should turn the sample from a blue to a yellow color and the samples should be read within 10 minutes of adding the stop solution (25).

Results

Table 1: Interpretation of ELISA results

Absorbance		**Visual Color**	**Interpretation**
450nm	**450/620nm**		
< 0.150	< 0.090	Clear to slight yellow	(-) below detectable limit
>/= 0.150	>/= 0.090	Pale to strong yellow	(+) specimen contains *Giardia* antigen

Discussion and Conclusion

Testing of mouse species to detect *Giardia* cyst wall protein 1 (CWP1) is more effective than detection by immunofluorescence. Multiple samples can be tested simultaneously, and the procedure is less labor intensive. Normal mice not infected with *Giardia* should test negative in the ELISA test. A positive result is a sign that the mice are shedding detectable amounts of

Giardia antigen. If mice show a positive result, it would show that solar disinfection using titanium dioxide films was ineffective and the hypothesis not supported.

A negative ELISA test would show that solar disinfection using titanium dioxide was effective in removing *Giardia* cysts from the water samples. Testing the water after disinfection is a sort of fail safe. If *Giardia* cysts are present after solar disinfection, then the hypothesis will not be supported, and the water should not be injected into mouse samples. In this case, tests would be done to change aspects such as sunlight exposure time in hopes of disinfecting the water before injecting the test subjects.

Results of this investigation could have life changing effects for populations of developing countries. Effective disinfection and an in vivo test on mouse models showing eradication of *Giardia* with the absence of *Giardia* antigen could lead to low cost water disinfection methods. Because water disinfection would more effective, giardiasis cases would be greatly decreased, and that would lead to a domino effect of decreasing malnutrition and other adverse effects of giardiasis.

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